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p50^{Cdc37} is a recently discovered gene which functions in the establishment of protein kinase signaling pathways by functioning in complex with molecular chaperone Hsp90. The proposed mode of function of Cdc37/Hsp90 complex is that Cdc37 targets intrinsically unstable kinases to the complex with Hsp90, and this transient interaction of newly synthesized kinases with the complex is necessary for their stabilization and/or folding and further activation. Through stabilization of key kinases, Cdc37 expression may be a pre-requisite for activation of the signaling pathways and, as a result, for proliferation. Cdc37 expression may be required for proliferative aspects of development and Cdc37 would be required for cell proliferation induced by oncogenes that utilize protein kinase signaling pathways in which Cdc37 participates.

To test this hypothesis, we created and analyzed a transgenic mouse model where Cdc37 expression is driven by MMTV promoter, directing the expression into breast epithelial cells, salivary and lacrimal gland. The effects of Cdc37 overexpression in mammary and salivary glands are generally consistent with Cdc37 having a positive effect on proliferation and reveal a novel collaboration with the c-myc oncogene in cellular transformation.

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ABSTRACT

$p50^{Cdc37}$ is a recently discovered gene which functions in the establishment of protein kinase signaling pathways by functioning in complex with molecular chaperone Hsp90. Biochemical and genetic data indicate that Cdc37/Hsp90 complex plays a central role in the establishment of pathways that are directly implicated in cell cycle promotion and transformation. The proposed mode of function of Cdc37/Hsp90 complex is that Cdc37 targets intrinsically unstable kinases to the complex with Hsp90, and this transient interaction of newly synthesized kinases with the complex is necessary for their stabilization and/or folding and further activation. Essentially all of the oncoprotein kinase components of the receptor tyrosine kinase signalling pathways working through the Ras activation - including c-src homologs, Raf-1, and Cdk4 and 6 - have been shown to require Hsp90 (and by inference Cdc37) function for their stability. In addition, there is indirect evidence that Hsp90/Cdc37 may be involved in stabilization not only of cytoplasmic, but also receptor tyrosine kinases involved in responce to environmental signals. The Ras signal transduction pathway is considered to be one of the most important pathways controlling and coordinating diverse signalling events that regulate cell growth and differentiation, and many components of this pathway were identified as oncoproteins. Through stabilization of key kinases, Cdc37 expression may be a pre-requisite for activation of the pathway and, as a result, for proliferation.

The emerged hypothesis that Cdc37 is required for proliferation predicts that Cdc37 expression would be required for proliferative aspects of development and that Cdc37 would be required for cell proliferation induced by oncogenes that utilize protein kinase signaling pathways in which Cdc37 participates.

To test this hypothesis, we created and analyzed a transgenic mouse model where Cdc37 expression is driven by MMTV promoter, directing the expression into breast epithelial cells, salivary and lacrimal gland, as well in some other organs. The effects of Cdc37 overexpression in mammary and salivary glands are generally consistent with Cdc37 having a positive effect on proliferation and transformation.

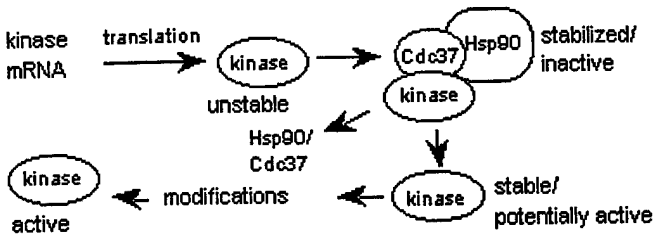
INTRODUCTION

An emerging theme in signal transduction is that the establishment of signaling pathways involving protein kinases requires the activity of a molecular chaperone that functions to stabilize newly synthesized protein kinases. The central component of this chaperone is Hsp90. This protein is physically associated with a variety of kinases, including Raf, Cdk4 and 6, and src homologs, and these kinase complexes also contain an additional p50 kDa subunit (1, 2, 3, 4, 18, 29, 49-51) of unknown identity and function. Moreover, genetic studies indicate that Hsp90 is required for the function of a variety of receptor and non-receptor tyrosine kinases in different organisms.

The association of pp60-src with cytoplasmic Hsp90 complex has been characterized in detail. Inactive pp60-src is found to be associated with Hsp90/p50 concomitant with or shortly after translation, and dissociation from the complex occurs with a half-life of 10 min. Active, membrane associated src lacks Hsp90/p50 (5, rev. in 1). In mammalian cells, pharmacological inactivation of Hsp90 through the action of herbimycin or geldanamycin leads to destabilization of a number of kinases, including Raf-1 (2), erbB2 (7-10), v-src (6), v-abl (15-16) and other src homologs (17), and in some cases - reversal of the transformed phenotype (19-25). These anti-tumor agents appear to function by binding to Hsp90 and blocking its ability to function as a kinase chaperone. Systemic or topical administration of the drugs markedly reduced the tumorigenecity of cell lines transformed by these kinases *in vivo* in nude mouse by decreasing activity of the critical tyrosine kinases (11-16). Thus, an emerging theme is that a potentially large number of kinases involved in proliferation and transformation are intrinsically unstable and require an Hsp90/p50 chaperone complex to facilitate the establishment of a functional signaling pathways.

We have recently identified the mammalian homolog of the budding yeast gene *CDC37*(26) as the p50 subunit of Hsp90/kinase complexes (3, 18). Based on our biochemical analysis, $p50^{Cdc37}$ functions as a protein kinase targeting subunit of Hsp90 and is required for association of Hsp90 with protein kinases (3). These results are supported by a variety of genetic data in both yeast and Drosophila, indicating a role for *HSP90* and/or *CDC37* in the kinase stability and function. Possible targets include *CDC28*, *MPS1*, *CKAI*, and *KIN28* in yeast, and *sevenless* and *torso* RTKs in flies (27, 28, 30-32, rev. in 3).

Our previous studies have been focused largely on the regulation of Cdk4, the catalytic subunit of D-type cyclin complexes (3). A substantial fraction of Cdk4 in fibroblasts is associated with a high molecular weight cytoplasmic complex containing Cdc37 and Hsp90. Biochemical data obtained suggest that release of Cdc37 occurs prior to the assembly of cyclin D/Cdk4 complexes. As with the other Hsp90 targets, pharmacological disruption of Hsp90 function leads to destabilization of Cdk4 and dissociation of the Cdk4/Cdc37 complex (3). Thus, Cdk4, like v-src, Raf-1, and erbB2, displays the characteristics of an intrinsically unstable protein that requires a chaperone for stabilization (Scheme 1).



Scheme 1. Proposed mode of action of Cdc37/Hsp90 complex in the stabilization of kinases. Unstable kinase is bound by Cdc37/Hsp90 shortly after translation. Kinase is inactive in the complex. Stabilized and potentially active kinase is released from the complex and after specific modifications become active and can participate in the signalling.

Involvement of Cdc37 in the stabilization of these kinases, directly implicated in cell cycle control and transformation, would predict that Cdc37 should be present in the actively dividing cells. Indeed, in situ analysis of Cdc37 expression in developing mice embryo shows that Cdc37 expression correlates with zones of high proliferation activity, supporting the notion that it may be required for proliferation. Cdc37 is an essential gene in Drosophila and budding yeasts, which is also consistent with the assumption that Cdc37 is required for cell viability and/or proliferation.

After releasing Cdc37, kinases can undergo additional modifications which allow them to be active. Such modifications can include phosphorylation, translocation or association with other subunits. D-type cyclins are regulatory subunits of Cdk4, kinsase which requires Cdc37 function for stabilization. Cyclin D1 has a specific role in the proliferation in the mammary gland. Induction of epithelial cell proliferation in mammary gland during pregnancy requires cyclin D1 (33, 34), and by inference, Cdk4. Given the involvement of Cdc37 in regulation of Cdk4 stability (8), we reasoned that Cdc37 may be required for proliferation of cells that require D-type cyclins and therefore might be coordinately regulated with cyclin D1 in this tissue. *In situ* hybridization revealed that Cdc37, like cyclin D1, is absent from the ducts of virgin mice but is massively induced during pregnancy (3). Other tissue types, which have descrete proliferative zones, such as small intestine and stomach, also display highly similar patterns of cyclin D1 and Cdc37 expression (3). These data are consistent with a positive role for Cdc37 in proliferation. In addition, we have recently found that normal prostatic epithelium does not express Cdc37. However, adjacent prostatic tumors have high levels of Cdc37 expression, consistent with the involvement of Cdc37 in proliferation and transformation. We decided to use the mouse mammary gland as a model to study the role of Cdc37 in development, proliferation and transformation in the breast.

Much of our current understanding of human breast development is derived from the use of animal models. The mouse mammary gland represents one of the best experimental systems available to study proliferation, development and transformation. Most of the development of the breast ductal system occurs after birth. This facilitates the use of a variety of experimental approaches to identify the molecules involved in the various steps of mammary development, including gene knockout and directed gene, tissue transplantation, hormonal and drug treatment and hormone withdrawal approaches, alone or in combinations. Recently, all these approaches have been successfully used to determine the developmental roles of hormones in the epithelial and stromal cells, and in epithelial-stromal interplay, to identify genes that play direct roles in the key aspects of mammary development, and to identify proteins that function as oncogenes when inappropriately expressed in the breast.

Mammary gland development in the mouse can be divided into several steps. An epithelial bud is the initial mammary structure formed at 12.5 dpc, followed by an epithelial sprout at 16dpc. In females, the epithelial sprout begins to grow into the fat pad during embryonic development under the influence of maternal hormones, and at the time of birth several ducts are present. During puberty in the female, these structures are the recipients of hormonal information that initiates the process of ductal elongation and sidebranching, which continues until the entire fat pad is filled. During periods of extensive growth in pregnancy, massive proliferation of lateral buds occurs and multiple alveoli are formed. After parturition, alveoli are major structures where producing and storing milk. Upon weaning, the bulk of the aveolar cells undergo programmed cell death to produce a ductal system reminiscent of that of a virgin. At each stage of mammary gland development, the events are driven by the combined action of steroid hormones and growth factors. Estrogen is required during puberty for ductal elongation, while progesterone and prolactin are required later for lateral bud development, alveologenesis, and lactation (52-56).

The mammary gland development in males is markedly different from that of the females. The epithelial bud normally undergoes an androgen-dependent partial destruction during embryogenesis. Typically, at birth male mice have only a rudementary ductal system consisting largely of an epithelial sprout with very short ducts, but unlike the situation with the female mice, this structure normally undergoes destruction during the first few weeks of birth (54).

Many available promoters will deliver expression of the target protein into epithelial cells of the mammary gland, and that enables the study of the role of the protein in different aspects of the mammary development and/or tumorgenesis. Different promoters will provide different temporal patterns of expression. Since the expression of Cdc37 is normally absent from the virgin breast, we wanted to direct its expression into virgin epithelium. MMTV promoter allows us to do it. MMTV promoter usually promotes the expression not only in the epithelial cells of mammary gland, but also salivary and lacrimal glands. Its expression is detected within several weeks after birth, increases at the onset of puberty, and increases further during pregnancy. In addition to the appropriate pattern of expression, this promoter provides an excellent opportunity to study the cooperative effect of the oncogenes, since multiple models utilizing this promoter are already available to study carcinogenesis in the breast and other organs expressing high levels of the transgene (35-48).

Our work seeks to understand the role of the Cdc37 gene in development and cancer. To begin addressing the role of Cdc37, we created MMTV-Cdc37 transgenic mice using a standard MMTV promoter and Cdc37 ORF. This report describes creation of the transgenic mice, developmental effects of Cdc37 expression in the breast and cooperative interaction with c-myc in induction of salivary gland tumors. These mice provide a novel system for studying some aspects of breast development, cellular transformation, and oncogene collaboration.

EXPERIMENTAL METHODS, ASSUMPTIONS AND PROCEDURES

Creation of MMTV-Cdc37 mice

Cdc37 open reading frame (ORF) was inserted under control of MMTV promoter (2.2 kb). To ensure the correct processing of the resulting pre-mRNA, bovine polyadenylation signal was added on the 3' end and rabbit β–globin splice site was inserted into the 5' untranslated region. These exogenous sequences also served to provide a probe which would recognize only exogenous mRNA product. Since the exogenous and endogenous mRNAs are of the very close size (1.6 kb), it provides an important tool in separating the two. The resulting protein was indistinguishable from its endogenous counterpart. Resulting DNA construct (Fig. 1A) was purified, and linear DNA was injected into the male pronuclei of the developing mouse zygote of the outbreed strain B6D2F1 x ICR. The injected zygotes were then transferred into the oviducts of the pseudo-pregnant recipient female and resulting pups were analyzed for the insertion of the transgene by Southern blotting of the tail DNA.

Mice strains and matings

The original strain of mice used for DNA injection was of the mixed background (B6D2F1x ICR). The resulting transgenic animals were continuously backcrossed with outbreed ICR strain. Non-transgenic littermates were used as the controls in all described experiments. My attempts to bring the lines to homozygosity were unsuccessful, as it is quite usual in cases of non-targeted insertion of heterogeneous DNA.

Southern

Tail DNA was isolated by the standard technique described somewhere else. 20 μg of total DNA were used for overnight restriction digestion with BamHI. Digested DNA samples were run on 1% agarose gel, transferred to the Hybond N+ (Amersham) filter in 0.4% NaOH, and hybridized with radiolabelled Cdc37 probe (random-primed ORF). Restriction with BamH1 allows differentiation between endogenous and exogenous Cdc37 fragments.

Northern

Total RNA from different tissues was extracted using Trisol reagent (Life Technologies, Inc.), resolved on the agarose gel and transferred to the nitrocellulose, which was blotted with nick-translated full lenght Cdc37 probe or with probe corresponding to rabbit and bovine sequences in the transgenic construct.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on the 5 micron sections made form formalin-fixed and paraffin-embedded tissues, using standard techniques. Secondary antibodies linked to fluorescin (FITC) or Cy3 (red fluorescence) were used. Haematoxylene and eosine (H&E) staining was performed on the adjacent sections.

Whole-mount analysis of the breast tissue

Whole-mount analysis of the breast tissue allows for the observation of the three-dimensional structure of the mammary gland. Selective stain technique stains epithelial cells of the breast ducts and visualizes them. Two methods are currently used for visualization. Stainings presented here are made using hematoxylene, staining with carmine alum gives less background and higher specificity and is currently used in the lab.

Hematoxylene staining

Fourth pair of the mammary glands (inguinal) is excised from the animal, fixed in 10% formalin overnight. After that, tissues are placed in acetone for 24-48 hours, and prepared for staining by 1 hour

washes with 100% and 95% ethanol. The breast is then spread are stained with hematoxylene stain (95% EtOH, 0.2% $FeCl_3$, 0.1% hematoxylene, 3% HCl) for overnight, and washed with water and increased concentrations of EtOH, 3 changes of xylene and stored in methyl salycilate in glass containers for viewing and photography.

Carmine Alum staining

Inguinal glands are spread on the glass and fixed for 4 hours in Carnoy's fix (60% EtOH, 30% chloroform, 10% glacial acetic acid) and stained over night in Carmin Alum stain (1g carmine, 2.5g aluminium potassium sulfate in 500ml of water, boiled for 20 min and filtered). Stained spreads are washed with increased concentrations of EtOH, twice with xylene and stored in xylene for further viewing and photography.

RESULTS AND DISCUSSION

Creation of MMTV-Cdc37 mice

After injection of the MMTV-Cdc37 purified DNA construct (Fig.1A) into mice zygotes and transplantation into recipient female, 45 pups were born. Two of the pups contained multiple inserts and passed the transgene to their progeny. These strains (MMTV-Cdc37.1 and MMTV-Cdc37.2) were analyzed further. Strain MMTV-Cdc37.1 contains about 15 copies of the transgene, and strain MMTV-Cdc37.2 has about 10, as estimated by the intensity of the Southern bands (Fig. 1B).

My attempts to bring both strains to homozygosity were unsuccessful, probably because the insertion of the transgene knocked out a portion of the genome with essential gene(s). It is not a unique problem with my transgenic animals, and this phenomena is common then constructing transgenic animals by non-homologous insertion of a DNA fragment. All our work is carried on the heterozygous transgenic animals, and their non-transgenic littermates are used as controls for all experiments described. Proper controls are very important since originally our transgenic animals were constructed on mixed background, and littermates constitute the best possible control.

Characterization of expression by northern analysis and IHC

Usually, MMTV promoter governs a high level of expression in the mammary epithelial cells, lacrimal and salivary glands. Occasionally, transgene is expressed in other tissues, most often testis, lymphatic tissues and other organs. To characterize the pattern of expression of Cdc37 in our two transgenic lines, northern blot analysis of multiple tissues was performed. Both strains express exogenous mRNA at high levels in the mammary, salivary and lacrimal glands (Fig.1C). Both strains expressed Cdc37 also in testis, and MMTV-Cdc37.1 also in the uterus. In general, the levels of expression in the MMTV-Cdc37.2 line were somewhat lower then in MMTV-Cdc37.1, probably reflecting the fact that the MMTV-Cdc37.2 line has fewer copies of the transgene.

Immunofluorescence analysis on tissue sections with affinity purified antibodies demonstrated that the Cdc37 transgene was expressed at high levels in the secretory epithelial cells of salivary gland ducts, in the Leydig cells of the testes, and in the ductal epithelial cells of the mammary gland (Fig. 2). Expression in the salivary gland and testis is largely uniform, with 85-95% of all cells expressing the transgene, while only about 10-15% of breast epithelial cells expressed Cdc37. Non-homogenous expression in the breast is a common characteristic of the MMTV-driven protein expression.

As I show below, male MMTV-Cdc37 mice of both transgenic lines, unlike their wild-type counterparts, develop a mammary ductal system, and immunofluorescence analysis reveals the expression of Cdc37 in the mammary epithelial cells in these mice in about the same percentage of the cells as in females.

Transgenic males develop breast ducts

Our phenotypic analysis was focused on the organs shown to express exogenous Cdc37. No abnormalities were found in the testis and the salivary gland, based on comparison of the organs of transgenic animals and their non-transgenic littermates.

In females, the initial bud formed on the day 16dpc undergoes further development *in utero*, while the bud in the males undergoes androgene-dependent partial involution. The extent of this involution is variable in different strains of mice. In our strain about 60-70% of 4 week-old males had an epithelial sprout with very short ducts in the inguinal pair of the mammary glands. These remnants of the epithelial sprout will generally recede after 4 weeks of birth, and only about 10% of 6 week old and older non-transgenic animals contain some epithelial sprouts.

In transgenic animals, the sprout is not destructed after the age of 4 weeks, but undergoes the proliferation and forms the extensive structures with side branches. About 70% of adult transgenic males in the MMTV-Cdc37.1 line have moderately or well-developed ductal system, although this development is much less extensive than in females (Fig. 3). The percentage of males who develop duct system in another strain, MMTV-Cdc37.2, is obout 30% in adults, possibly reflecting the lower

level of expression of the transgene in this line. The effect observed in MMTV-Cdc37 males is unique and was never observed before.

The effect of Cdc37 in male mammary gland development is consistent with the general positive effect Cdc37 should have on proliferation. Its exact mechanisms are not known and should be investigated further in the coming year.

Transgenic females show increased proliferation during pregnancy

Mammary gland development in the mouse begins with a formation of an epithelial bud which is generated at 12.5dpc, and formation of an epithelial sprout follows at day 16dpc. In females, this epithelial sprout undergoes some proliferation under the influence of maternal steroid hormones, and presents at birth as an initial structure with several ducts and branches. This structure begins to grow again after hormonal stimulation initiates the processess of ductal elongation and side-branching at the onset of the puberty (6 weeks after birth), and by 8-9 week fills the fat pad. During pregnancy, extensive proliferation of sidebranches occurs, and gives rise to lateral buds which differentiate into milk-producing alveoli upon parturition.

MMTV-Cdc37.1 transgenic mice show increased density of the lateral buds during pregnancy (Fig. 4A), which suggests that high levels of Cdc37 expression promote normal pregnancy-associated proliferation.

Massive proliferation during pregnancy is dependent on the cyclin D1 and is absent in cyclin D1 knock-out mice. Our previous results show that both cyclin D1 and Cdc37 are absent in the virgin breast and are induced during pregnancy. Cyclin D1 is required for the activation of Cdk4 protein kinase, which is shown by us previously to require Cdc37 for its stability. The possible mode of action in the situation would be that increased level of Cdc37 cooperates with cyclin D1 in formation of the active Cdk4/cyclin D1 complexes, which in their turn promote lateral bud development.

I will continue to study this effect, in part by crossing the mice with MMTV-cyclin D1 to see if I will observe the increase of cooperativity.

Transgenic females have delayed involution after lactation

Upon weaning, the bulk of the alveolar cells undergo apoptotic cell death to produce a ductal system reminiscent of that prior to pregnancy. When the whole-mount preparations of the inguinal mammary gland from transgenic and non-transgenic females on days 15 after weaning of the pups are compared, the difference in the epithelial cells density in involuting alveoli becomes obvious. Transgenic animals have more epithelial cells in the alveolar area, and the more dense alveolar structures. On the day 21, there is no difference between transgenic and non-transgenic animals in the alveolar involution, but transgenic females have more lateral buds, that is probably reflecting the fact that they acquired more of them during pregnancy-related proliferation burst (Fig.4A).

To confirm and characterize this delay in involution more precisely, I decided to look at the earlier phases of the involution process. On the day 3 after weaning, control non-transgenic females have completely collapsed alveoli which already lack milk, while transgenic MMTV-Cdc37.1 animals have alveoli which are not collapsed and contain a significant amount of milk. Alveoli of transgenic animals at 5 days still contain some milk-like substance and just have initiated the process of collapse (Fig. 4B).

More studies are under way on this phenomenon, such as determination of the level of Cdc37, cyclin D1 and Cdk4 in these cells, determination and comparison of the rates of cell proliferation and apoptosis in transgenic and control animals.

Crosses of MMTV-Cdc37.1 line with MMTV-neu, myc, cyclin D1

Our original hypothesis proposed that Cdc37 could cooperate with other oncogenes in induction of the transformation of mammary gland. In order to test this hypothesis, I crossed mice expressing Cdc37 with mice expressing one of the following oncogenes: neu (ErbB2), myc and cyclin D1. All of these oncogenes are inducing mammary gland tumors in adult animals. Mice bearing Cdc37 with neu and myc oncogenes are now about 9 months old and just started to develop mammary tumors. So far, only few tumors are observed, and no cooperating effect is seen so far in mammary gland

transformation, but the number of tumors is very small by now (2-3 in each group of oncogene alone or Cdc37/oncogene combination), no conclusions can be reached as yet.

Mice bearing double transgene with cyclin D1 are only 1.5 months old, and cyclin D1 mice by itself develop tumors after 1 year of age. In crosses with cyclin D1 we are looking not only for cooperation effect in terms of induction of mammary gland or other kinds of tumors, but also for the effect of overexpression of the double transgene on the pregnancy-related extensive proliferation and post-weaning involution of the alveoli. These studies are in the beginning stage now and are to be continued.

Cooperation with myc in salivary gland tumor induction

MMTV-driven oncogenes are known to induce a wide array of the tumors, not only mammary-specific. The other places of high expression are also known to give rise to the tumors. MMTV-Ras mice develop salivary, lacrimal (Harderian) and mammary gland tumors at similar rates. Neu mice develop predominantly mammary adenocarcinoma and occasionally other kinds of tumors.

The rates of induction of mammary tumors in mice bearing MMTV-myc and MMTV-neu transgenes are very close. Age at which 50% of the animals develop breast tumors are 325 and 205, respectively for these oncogenes. About 5% of MMTV-neu adult mice develop a salivary gland tumors. MMTV-myc mice, even though they do express the oncogene in salivary glands, are not known to develop this kind of tumors, and this fact is well documented and is considered a peculiarity of the myc oncogene. No other proteins which would cooperate with myc in induction of salivary gland tumors are known, although gene products cooperating in other kinds of myc-induced tumorgenesis are described.

Four out of 25 mice, bearing both MMTV-Cdc37 and c-myc oncogene, had developed salivary gland tumors (Fig.5A) by the age of 9 months. None of the single transgenics (Cdc37 alone or c-myc alone) developed any salivary gland tumors (MMTV-Cdc37 lines were monitored for more than a year). Although the numbers are low so far, the salivary tumors represent considerable portion of all tumors developed, and I saw 4 salivary gland tumors vs 2 breast tumors in the same population of Cdc37/myc double transgenics. As I started to find these tumors only recently, I expect the percentage of tumor-bearing animals will rise significantly. The rate of salivary tumor appearance is higher than the rate of appearance of the mammary gland tumors, and one of the animals having both kinds of tumors. These salivary tumors were analysed by Dr. Finegold (Texas Childrens Hospital) and found to be salivary adenocarcinomas.

The kind of salivary tumors induced by simultaneous expression of Cdc37 and myc are similar to the kind of tumors induced by MMTV-Ras in the salivary gland. Both have very similar morphology, are rapidly progressing and have high mitotic indexes. This observation lead to speculation that expression of Cdc37 in double transgenics can help to reveal some mutations which lead to activation of the Ras signaling pathway. This can be achieved by Cdc37-mediated stabilization of key kinase in pathway which would reveal spontaneous mutations, that would otherwise not lead to transformation.

I will attempt to discover mechanisms responsible for cooperative effects of simultaneous expression of Cdc37 and c-myc by checking the tumors for mutations in genes commonly mutated in different kinds of cancer such as p53, Ras, deletion of p16/ARF region. I will also check for activation of the Ras pathway and stabilization of key kinases from this pathway.

Creation of PB-Cdc37 mice and invasive transformation of the prostate in these mice

Cdc37 expression is absent in normal human prostate epithelium but is highly induced after transformation. Interestingly, Cdc37 induction is apparent in some pre-malignant lesions, probably reflecting the fact that Cdc37 induction is a early event in transformation. To test the effect of Cdc37 expression in normal prostate, Cdc37 ORF was placed under control of PB (probasin) promoter which directs expression selectively to the prostate. SV40 splice site and polyadenylation sequences were added to ensure the proper post-translational processing of the mRNA. Several lines of transgenic animals were generated, and inheritability of the insert was ensured. Prostates of transgenic and control non-transgenic animals were fixed, embedded in paraffin, sectioned, stained with H&E and analyzed under the microscope. One of the 4 animals examined from one of the lines of transgenics had

developed an unusual invasive transformation in the prostate (Fig 5B). Analysis of the pattern of expression of the transgene in the animals and particularly, Cdc37 in the transformation zone, is under progress now and will be reported later.

CONCLUSIONS

Since the time the grant was awarded, we made significant progress in constructing MMTV-Cdc37 and PB-Cdc37 mice, analyzing the expression pattern of the transgene and describing the phenotype resulting from Cdc37 expression. Highest levels of expression of the transgene is observed in mammary, salivary and lacrimal glands. Transgenic males develop extensive breast duct system and thus displaying unique phenotype. Transgenic females show higher proliferation in the breast during pregnancy and delayed involution of the alveolar structures after weaning. Another unique phenotype is demonstration of oncogenic potential of Cdc37 in cooperation with myc in inducing salivary gland adenocarcinomas and then expressing Cdc37 in prostate of PB-Cdc37 mice. In general, our observations are consistent with Cdc37 playing important role in development, proliferation and carcinogenesis. It would be of great importance to determine exact mechanisms of oncogenic action of Cdc37 and this is the goal of my future work.

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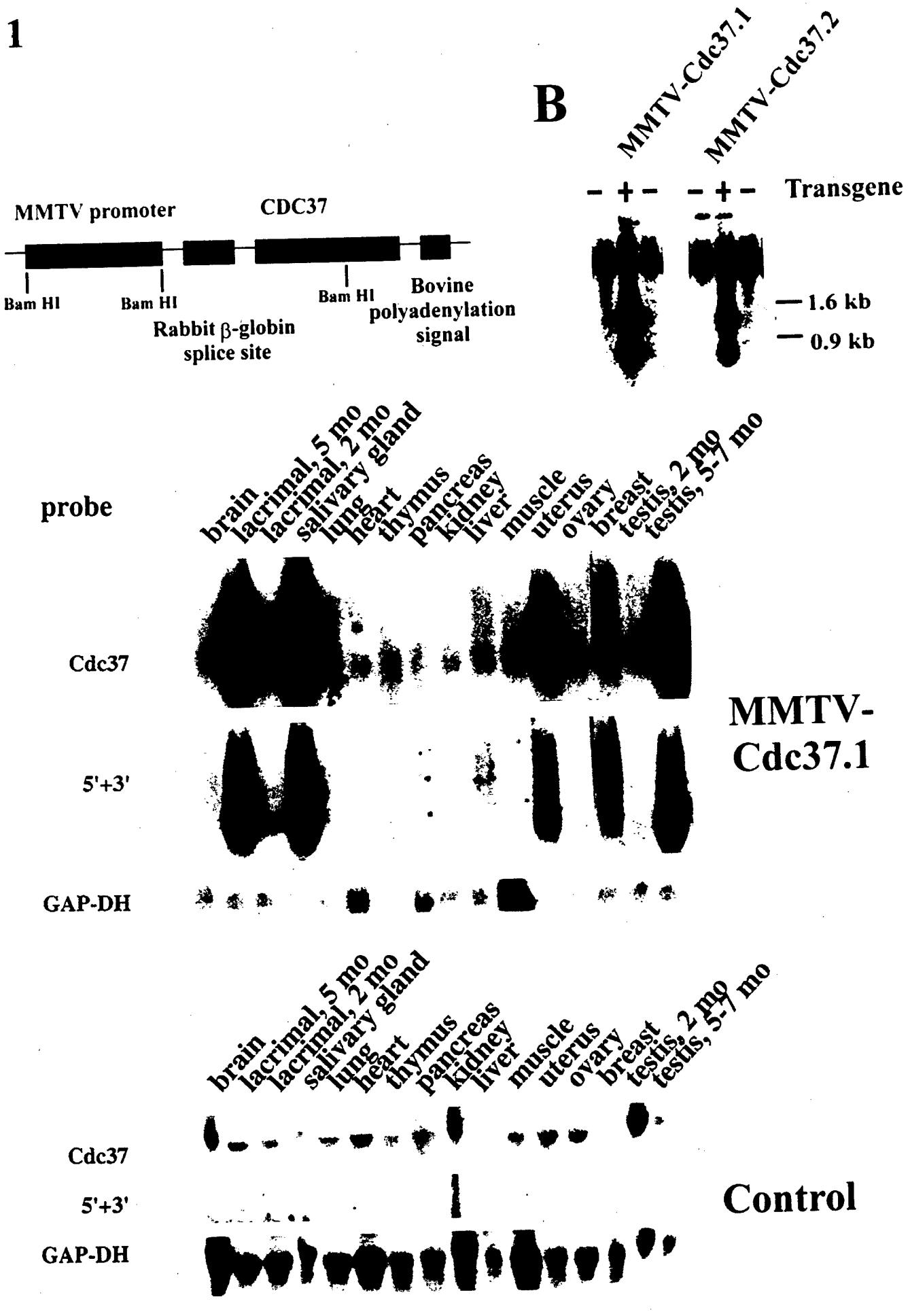
APPENDICES

Fig. 1


A
MMTV promoter
CDC37
Bam HI
Bam HI
Bam HI
Rabbit β-globin splice site
Bovine polyadenylation signal
B
MMTV-Cdc37.1
MMTV-Cdc37.2
- + - - + -
Transgene
1.6 kb
0.9 kb
C
probe
brain
lacrimal, 5 mo
lacrimal, 2 mo
salivary gland
lung
heart
thymus
pancreas
kidney
liver
muscle
uterus
ovary
breast
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Cdc37
5'+3'
GAP-DH
MMTV-Cdc37.1
Control

Fig. 2 Cdc37 expression in transgenic animals

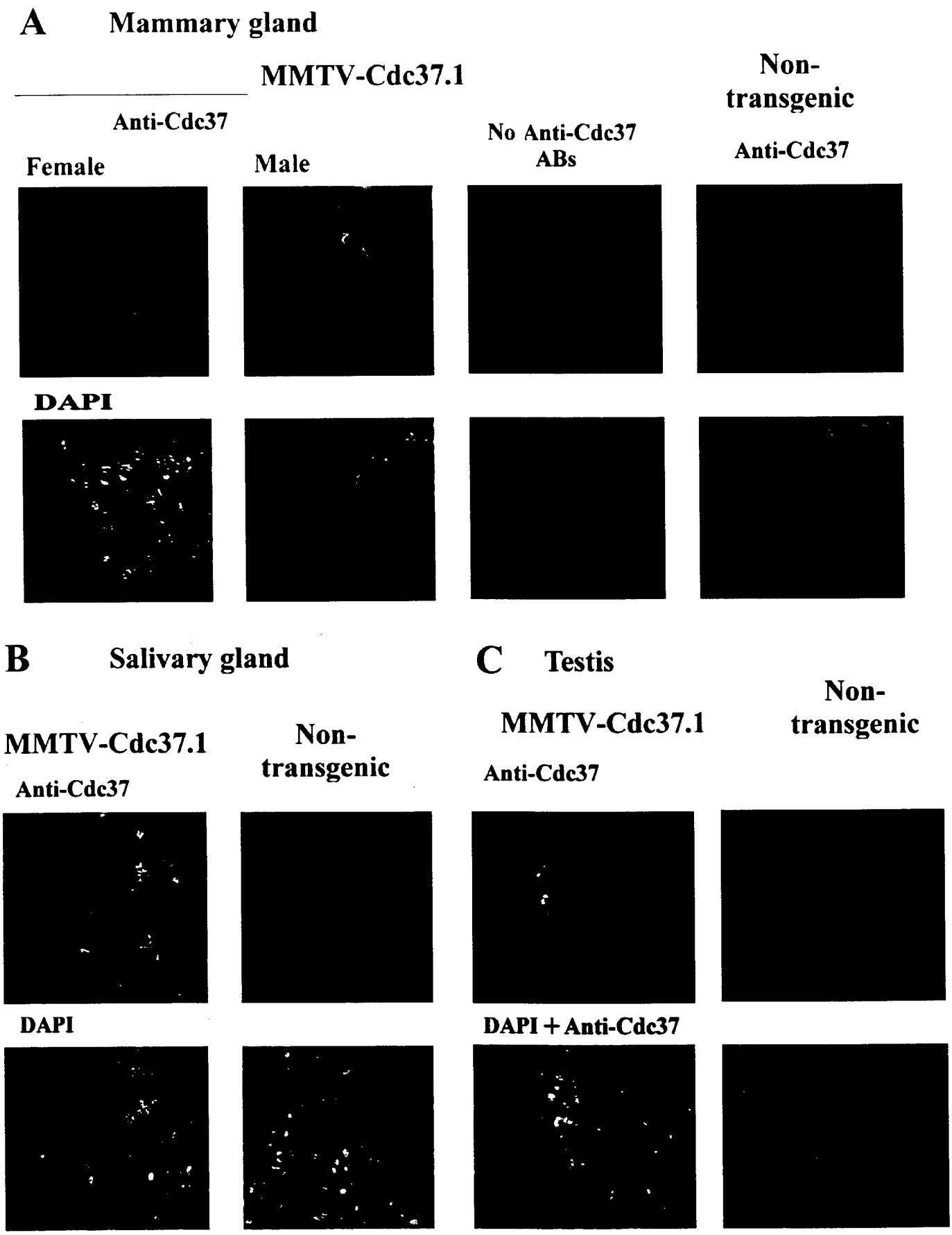

Fig. 3 Transgenic males develop breast ducts

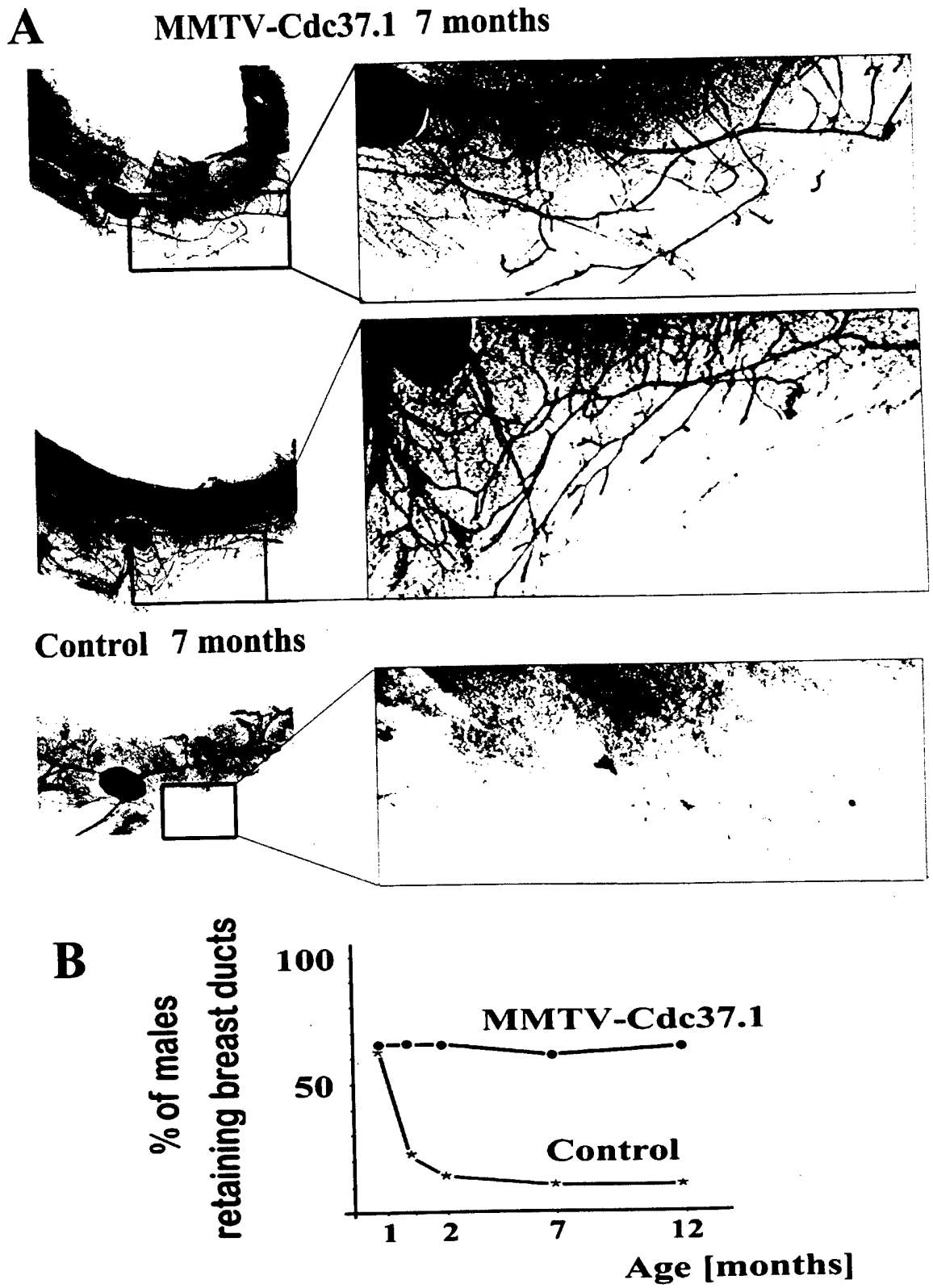

Fig. 4

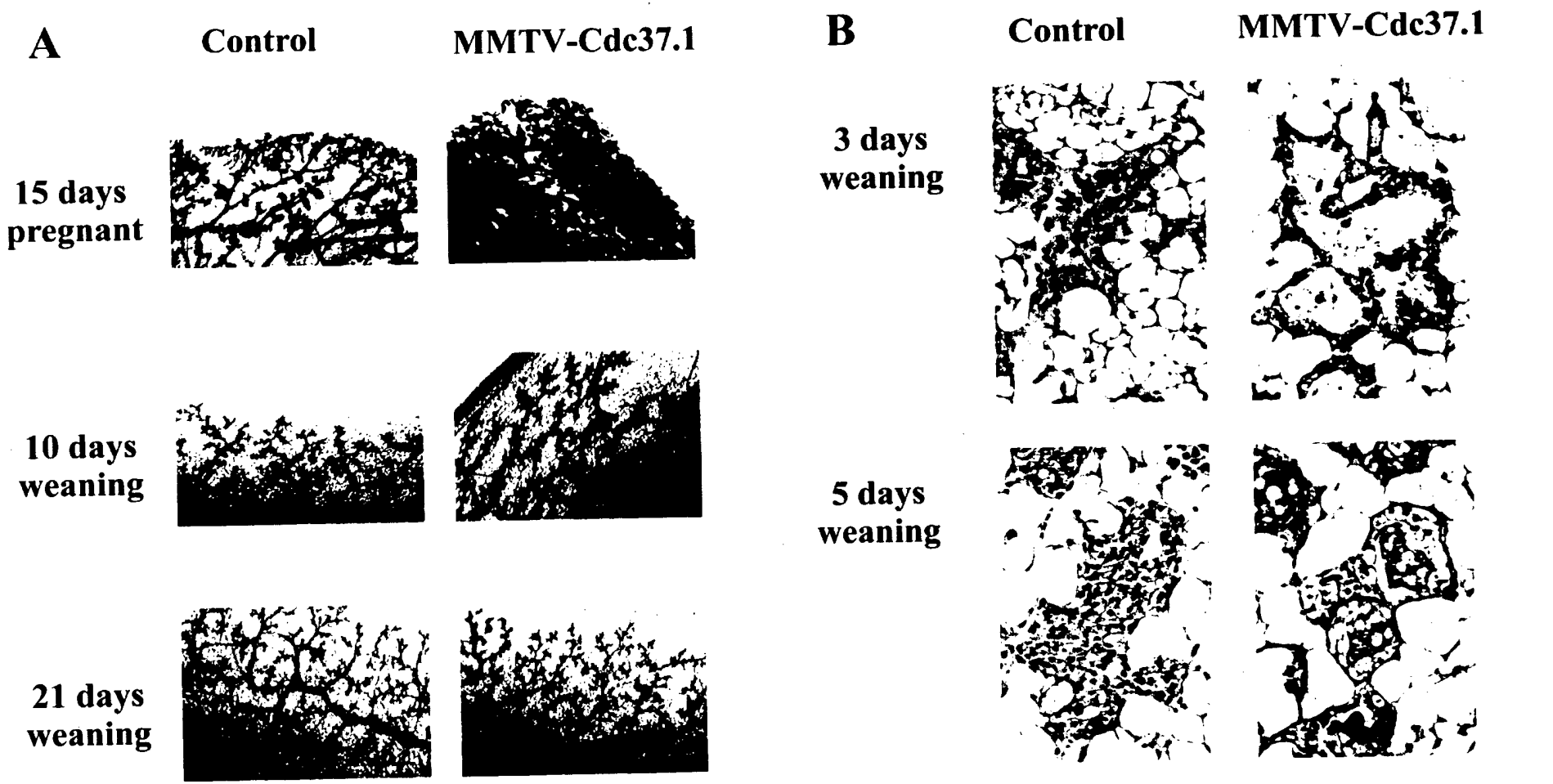
A
Control
MMTV-Cdc37.1
15 days pregnant
10 days weaning
21 days weaning
B
Control
MMTV-Cdc37.1
3 days weaning
5 days weaning

Fig. 5 Oncogenic transformation in mice expressing Cdc37